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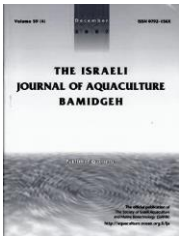

Effects of Low Salinity on Growth, Survival, and Stress Response in the Longtooth Grouper, *Epinephelus bruneus,* and *E. bruneus* × *E. lanceolatus* Hybrid Grouper

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Keywords: giant grouper; growth; hybrid; longtooth grouper; salinity

Abstract

This study investigated the effects of low salinity on growth, survival, and stress response in longtooth grouper, *Epinephelus bruneus,* and the hybrid grouper resulting from crossing *E. bruneus* females and *E. lanceolatus* males. There were no significant differences in the hybrids for any of the above growth values among different salinity groups, however the percent weight gain was significantly lower in the 8 Practical Salinity Unit (psu) group than in the other salinity groups. As for survival, only longtooth grouper in the group reared at 8 psu died. The plasma cortisol levels tended to increase at lower salinities in both the longtooth grouper and the hybrids. Plasma Na^+, K^+, Cl^- concentrations in the hybrid were not significantly different among the salinity groups, while Na^+ and Cl^- concentrations in the longtooth grouper were significantly lower in the 8 psu group than in the other treatments. The above results indicate that both the longtooth grouper and the hybrid grouper juveniles were highly adaptable to variations in salinity. The optimal salinity for rearing longtooth grouper was found to be 24 psu, while the hybrids had a larger tolerance range of 16-32 psu. Results therefore showed that the hybrid grouper has a higher tolerance than the longtooth grouper for low salinity.

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Introduction

Global farm production of fish from the family Serranidae reached approximately 118,039 tons at 2012. Most fish production comes from China and Southeast Asian countries and approximately 50% is consumed in China (FAO, 2014). Serranids are considered high-end species, earning high prices on the global fish market, but the recent rapid decline in wild stock has led to catch reduction. Among the serranids, 20 commercially valuable species are considered endangered species on the International Union for Conservation of Nature (IUCN) Red List, indicating that there is an urgent need to implement conservation measures for those species. Although serranid species are produced in Taiwan, China, South Korea, and some southeast Asian countries, supply is much lower than demand. This is due to low survival rates resulting from a lack of data on the reproductive biology, the small mouth size of hatched larvae, the high rate of cannibalism, and viral diseases.

Among the species inhabiting Korean waters is the longtooth grouper, *Epinephelus bruneus* (family Serranidae, order Perciformes), found on coral reefs, rocks, and muddy areas in subtropical and tropical waters. In Korea, there are eight serranid species, including *E. bruneus*, *E. akaara*, and *E. septemfasciatus*, which inhabit the southern coast of the peninsula and the coastal waters of Jeju Island (Kim and Lee, 1994). The longtooth grouper can survive the winter in Jeju and some of the southern waters of Korea. With the available technology for seed production and rearing, a limited amount of farming is conducted in Jeju. However, due to slow growth before these fish attain market size, farms refrain from producing them in mass. Another serranid species, *E. lanceolatus*, has recently attracted interest for farming in China, Taiwan, and some Southeast Asian countries. These fish live in the tropical coastal waters of the Indian and Pacific Oceans and are known to grow up to 270 cm in length and 400 kg in weight (Pauly and Froese, 2014; Fishbase, 2017). Despite its rapid growth and high tolerance for warm temperatures, its low cold tolerance makes it difficult to farm *E. lanceolatus* in areas with cold winters. Therefore, it is desirable to develop a new serranid species that can be farmed in areas such as Korea, with cold winter waters. In an attempt to overcome this problem, we hybridized female *E. bruneus* with male *E. lanceolatus*. The hybrids exhibited faster growth than the pure longtooth grouper and higher cold tolerance than the pure *E. lanceolatus* during the juvenile stage (data not shown). To introduce the hybrid as a new species for farming, its adaptation to salinity among other aspects must also be considered. Salinities of culture water can affect the osmotic regulation of marine fish thereby causing disruptions in the ion and water equilibrium, impacting on physiological conditions, and delaying growth (Boeuf and Payan, 2001). The effects of salinity on fish growth are well described in Boeuf and Payan (2001). There are also numerous studies on the effects of variations in salinity on the physiological characteristics and growth of bony fishes. As for serranid fishes, studies have been conducted on the effects of sudden changes in salinity (Tsui et al., 2012; Cheng et al., 2013; Garcia et al., 2013; Chapman et al., 2014; Othman et al., 2015; Sutthinon et al., 2014; Lim et al., 2016). Despite the increasing importance of aquaculture, there is still a paucity of data related to the effects of variable salinities.

Drastic changes in salinity (e.g., brackish water in an estuary, flooding, etc.) in limited areas of fish farming affect the blood composition of farmed fish, causing respiratory distress and eventual death. Variable salinities also impose chronic stress on fish, leading to retarded growth, diseases, and reduced immunity (Min et al., 2006). We therefore sought to demonstrate the effects of variations in salinity on the growth and survival of the hybrid serranid obtained by crossing female *Epinephelus bruneus* and male *E. lanceolatus* and investigated stress response at lower salinities in the hybrid.

Material and Methods

Experimental fish and rearing conditions

We used juvenile longtooth grouper and hybrids, produced in 2015 jointly by the aquaculture research lab of Mokpo National University and the Chungsol Hatchery located in Muan-gun, Jeollanam-do, Republic of Korea. The initial length and weight of the longtooth grouper was 8.9 ± 0.1 cm and 10.7 ± 0.3 g respectively, and for the hybrids 10.6 ± 0.1 cm and 20.3 ± 0.7 g respectively. Four rectangular fiber-reinforced plastic (FRP) tanks measuring 270 × 110 × 60 cm, and an operational water capacity of 1.5 m^3 were used with two replicates of a set of two square cages (50 × 50 × 50 cm) that were

installed in each tank. Thirty juveniles were stocked and reared in each cage. The water temperature was 25 ± 1.0°C and light-dark photo period was natural. Water salinity was gradually decreased from 32 practical salinity units (psu) at a rate of 8 psu per day in all four tanks until reaching a final salinity of fixed levels of 32 (control), 24, 16, and 8 psu. The fish were reared for 40 days. To exchange the tank water, we filled a reservoir pond with water treated for the appropriate salinity and temperature conditions. On a daily basis, the entire tank water was exchanged (1.5 ton/day). Half of the top of each tank was covered with a screen to stabilize the fish. A commercial formulated feed for olive flounder (ϕ2.9–3.4 mm, Wooseung No. 7, Daejeon, Korea) was administered three times a day, and waste on the tank bottom was siphoned daily.

Sampling and analyzing fish blood

Ten animals were randomly collected from each experimental group at the beginning and the end of the experiment. Blood collection was carried out with a heparin sodium prefilled syringues (20 IU/mL, Choongwae Co., Seoul, Korea). The sampled fish were anesthetized for 1 min with 150 ppm 3-aminobenzoic acid ethyl ester (Sigma-Aldrich, St. Louis, MO, USA). Within 1 min of anesthetization, blood was collected from the caudal peduncle from each fish. The collected blood samples were placed in 1.5mL microtubes, centrifuged, and then stored at -80°C until further analysis.

Plasma osmolality was measured with an osmometer (Vapor Pressure Osmometer Model 5520, Wescor, Logan, USA). Plasma Na^+, K^+, and Cl^- concentrations were measured with an automatic hematology analyzer (i-Smart 30 Vet Electrolyte Analyzer, i-SENS, Seoul, South Korea) and plasma glucose was measured with an automated biochemical analyzer (FUJI DRI-CHEM 4000i, Tokyo Japan). Plasma cortisol was measured by enzyme immunoassay (EIA) using the cortisol EIA kit (Oxford Biomedical Research, Oxford, USA).

Growth and survival rates

The amount of feed supplied daily was recorded from the beginning to the end of the experiment. Total length and weight of the fish were measured to the nearest 0.1 cm and 0.1 g, respectively, with vernier calipers and an electronic scale. Using the measurements, the following equations were used to calculate growth factor values. The number of dead animals was counted daily and used to calculate the survival rate.

Weight gain, $G\ (\%) = (W_2 - W_1)/W_1 \times 100$

Specific growth rate, $I\ (\%) = [\ln(W_1) - \ln(W_2)] \times 100/D$

Daily feed intake, $B\ (\%) = F \times 100/[(W_1 + W_2 + W_3) \times D/2]$

Feed efficiency $(\%) = (G/F) \times 100$

Condition factor $(\%) = W_2/L^3 \times 100$

Where B= Daily feed intake; D= Rearing days; F= Total feed supply; G= Weight gain; I= Specific growth rate; W_1= Total fish weight at the start of rearing; W_2= Total fish weight at the end of rearing; W_3= Total weight of dead fish during rearing; L= Total fish length at the end of rearing.

Statistics

Significant differences among the measurements (mean ± SD) were tested by Tukey's post hoc test in conjunction with analysis of variance (AVOVA) using SPSS statistical software version 23 (SPSS, Chicago, IL, USA). Significance was set at $P < 0.05$.

Results

Effects of salinity variation on growth and survival

The effects of salinity variation on *E. bruneus* weight increase, percent weight gain, specific growth rate, feed efficiency, daily feed intake, and condition factor are shown in Table 1. The average total length of the longtooth grouper was 8.8 ± 0.1 cm at the start of the experiment. No significant differences were found among the experimental groups. Weight, which increased to 20.3 ± 0.9 g in the 24 psu group by the end of the experiment was significantly higher than in the other groups. The percent weight gain

exhibited a similar trend to weight increase and was significantly higher in the 24 psu group than in the other groups, at 89.0 ± 8.5%. Specific growth rate was also the highest in the 24 psu group, at 1.6 ± 0.1%. Feed efficiency was at 80.5 ± 3.6% and 78.0 ± 5.7% in the 8 and 24 psu groups, respectively and significantly higher than in the 16 and 32 psu groups. Daily feed intake was significantly lower in the 8 psu group, at 1.5 ± 0.0%, than in the other groups. The condition factor did not differ significantly among the experimental groups.

Table 1. Growth performance of juvenile longtooth grouper *Epinephelus bruneus* reared at different salinities during the experimental period.

Salinity (psu)	*8*	*16*	*24*	*32*
Initial total length (g/fish)	8.8±0.1			
Final total length (g/fish)	10.8±0.1	11.2±0.1	11.4±0.2	10.9±0.03
Initial body weight (g/fish)	10.7±0.7			
Final body weight (g/fish)	17.5±0.6^{b}	17.9±0.7^{b}	20.3±0.9^{a}	17.3±0.4^{b}
Weight gain (%)	63.3±5.7^{b}	66.9±6.5^{b}	89.0±8.5^{a}	61.1±3.5^{b}
Specific growth rate (%)	1.2±0.1^{b}	1.3±0.1^{ab}	1.6±0.1^{a}	1.2±0.1^{b}
Feed efficiency (%)	78.0±5.7^{a}	59.1±7.2^{b}	80.5±3.6^{a}	61.5±4.9^{b}
Daily feed intake (%)	1.5±0.01^{b}	2.1±0.1^{a}	1.9±0.04^{a}	1.9±0.1^{a}
Condition factor (%)	1.4±0.002	1.3±0.1	1.4±0.02	1.3±0.02

The values are mean±SD (n=30). Means within each item followed by the same alphabetic letter are not significantly different ($P>0.05$).
Weight gain: $[(W_2- W_1)/ W_1\times100]$.
Specific growth rate: $[\ln (W_2)-\ln (W_1)]\times100/ D$.
Feed efficiency: $(G / F)\times100$.
Daily feed intake: $F \times100/[(W_1+ W_2+ W_3)\times D /2]$.
Condition factor: $(W_2/ L^3)\times100$.
D: days reared, F: feed intake, G: wet weight gain, W_1: initial body wt, W_2: final body wt, W_3: dead fish wt, L: total length.

The average total length of the hybrid grouper in each group ranged from 15.4-15.6 cm by the end of the experiment, with no significant differences among the groups. The average weight at the end of the experiment was 56.4−60.4 g, which was about 3 times higher than the weight of 20.6 g at the start of the experiment, but there were no significant differences among the experimental groups. Percent weight gain increased to 193.6 ± 7.3%, in the 24 psu group and was not significantly different from the 16 and 32 psu groups, however it was significantly higher than the 8 psu group. In contrast to the longtooth grouper, the hybrids did not display significant differences among the different salinity groups in terms of specific growth rate, feed efficiency, daily feed intake, or condition factor (Table 2).

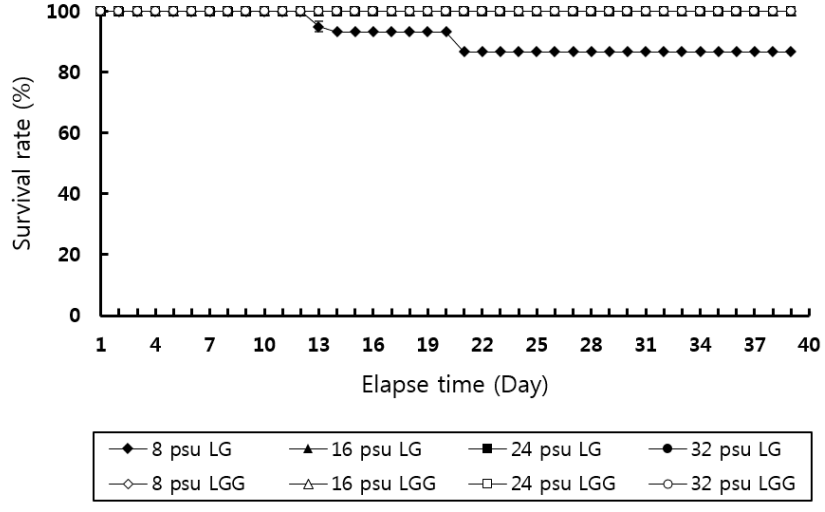
Table 2. Growth performance of juvenile hybrid grouper *E. bruneus* ♀ × *E. lanceolatus* ♂ reared at different salinities during the experimental period.

Salinity (psu)	*8*	*16*	*24*	*32*
Initial total length (g/fish)	10.6±0.1			
Final total length (g/fish)	15.4±0.3	15.5±0.2	15.6±0.1	15.6±0.2
Initial body weight (g/fish)	20.6±0.3			
Final body weight (g/fish)	56.4±3.3	59.4±1.1	60.4±1.4	59.6±1.3
Weight gain (%)	$174.2±16.0^{b}$	$189.1±5.6^{ab}$	$193.6±7.3^{a}$	$189.9±6.3^{ab}$
Specific growth rate (%)	2.5±0.1	2.7±0.1	2.7±0.1	2.7±0.1
Feed efficiency (%)	111.8±11.4	119.2±1.1	111.0±1.2	113.1±6.6
Daily feed intake (%)	2.1±0.1	2.0±0.02	2.2±0.02	2.2±0.1
Condition factor	1.6±0.01	1.6±0.02	1.6±0.00	1.6±0.04

The values are mean±SD (n=30). Means within each item followed by the same alphabetic letter are not significantly different ($P>0.05$).
Weight gain: $[(W_2- W_1)/ W_1×100]$.
Specific growth rate: $[\ln (W_2)-\ln (W_1)]×100/ D$.
Feed efficiency: $(G / F)×100$.
Daily feed intake: $F ×100/[(W_1+ W_2+ W_3)× D /2]$.
Condition factor: $(W_2/ L^3)×100$.
D: days reared, F: feed intake, G: wet weight gain, W_1: initial body wt, W_2: final body wt, W_3: dead fish wt, L: total length.

There were no dead hybrid groupers observed in any of the experimental groups during the 40-day period, and thus the survival rate of the hybrids was 100%. As for the longtooth grouper, there was no mortalities in the 32, 24, or 16 psu groups, but in the 8 psu group, dead animals were observed on days 13 and 21. The final survival rate was 86.7 ± 0.0 % (Fig. 1).

Fig. 1. Changes in survival rate (%) of juvenile longtooth grouper *Epinephelus bruneus* (LG) and hybrid grouper *E. bruneus* ♀ × *E. lanceolatus* ♂ (LGG) reared at different salinities.



Effects of salinity variation on plasma cortisol and glucose

The plasma cortisol and glucose levels of the longtooth grouper and the hybrids are given in Fig. 2. The plasma cortisol level of the longtooth grouper was the lowest in the 32 psu group, at 10.3 ± 6.6 ng/mL, and 8 psu group at 39.5 ± 0.0 ng/mL significantly higher compared to the other groups with ($P < 0.05$), showing a tendency for lower plasma cortisol levels at increased salinities. The cortisol levels of the hybrids displayed a similar tendency to that of the longtooth grouper, with the lowest level, 3.9 ± 2.8 ng/mL, at 32 psu, and a significantly higher level, 27.3 ± 4.7 ng/mL, at 8 psu, compared to

other salinity conditions. There were no significant differences in the plasma cortisol levels of the longtooth grouper and the hybrids among the different salinity conditions, except for at 8 psu.

The plasma glucose levels of longtooth grouper were lowest, 22.5 ± 0.5 mg/100 mL, at 32 psu, and highest, 32 ± 0.0 mg/100 mL, at 16 psu, indicating a significant difference between the 16 psu and the 24 or 32 psu groups. The plasma glucose levels of the hybrids were lowest, 11.5 ± 1.5 mg/100 mL, at 32 psu, and highest, 46.5 ± 0.5 mg/100 mL, at 24 psu. The plasma glucose levels were significantly higher at 24 psu than 32 psu in both the longtooth grouper and the hybrids.

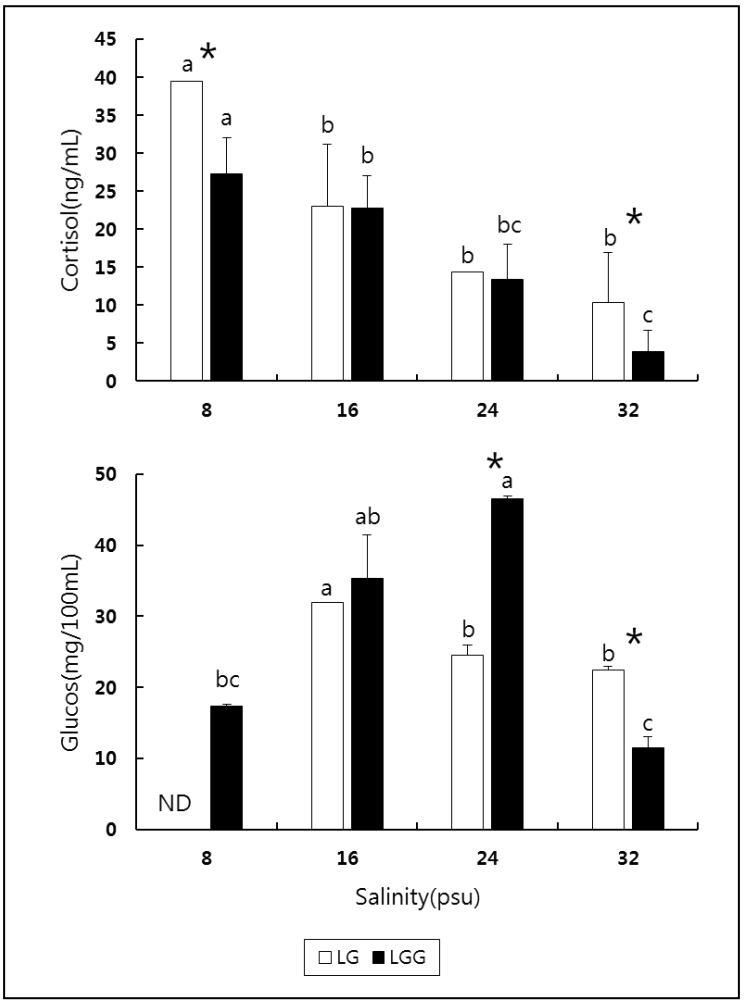


Fig. 2. Variations in plasma cortisol and glucose levels in juvenile longtooth grouper (LG) and hybrid grouper (LGG) at different salinities. Different superscripts indicate significant differences at different salinities ($P < 0.05$). Asterisks show significant differences between juvenile longtooth grouper (LG) and hybrids (LGG) at each salinity ($P < 0.05$).

Effects of salinity variation on Na^+, K^+, and Cl^- concentrations and osmolality

The plasma Na^+ concentration of the longtooth grouper was lowest, 118.3 ± 2.5 mmol/L, at 8 psu, and significantly higher (150 ± 5−160 ± 3 mmol/L) at 16, 24, and 32 psu. The Na^+ concentration of the hybrid grouper was highest, 168 ± 0 mmol/L, at 32 psu, but there were no significant differences among the experimental groups. Comparing the two species, the hybrids had significantly higher plasma Na^+ concentrations at 8 and 32 psu. The plasma K^+ concentration of the longtooth grouper was highest, 18.5 ± 1.6 mmol/L, at 8 psu, exhibiting a significant difference from the level at 32 psu. On the other hand, the hybrids exhibited no significant differences in plasma K^+ concentrations among all salinity groups. Comparing the two species, the longtooth grouper had significantly higher K^+ concentrations in each of the salinity groups. The plasma Cl^- concentration of the longtooth grouper was 100.7 ± 6.5 mmol/L in the 8 psu group, which was significantly lower than in the 16, 24 and 32 psu groups. However, the hybrids did not show any significant differences in Cl^- concentrations among the different salinity groups, similar to the results for the plasma Na^+ and K^+ concentrations.

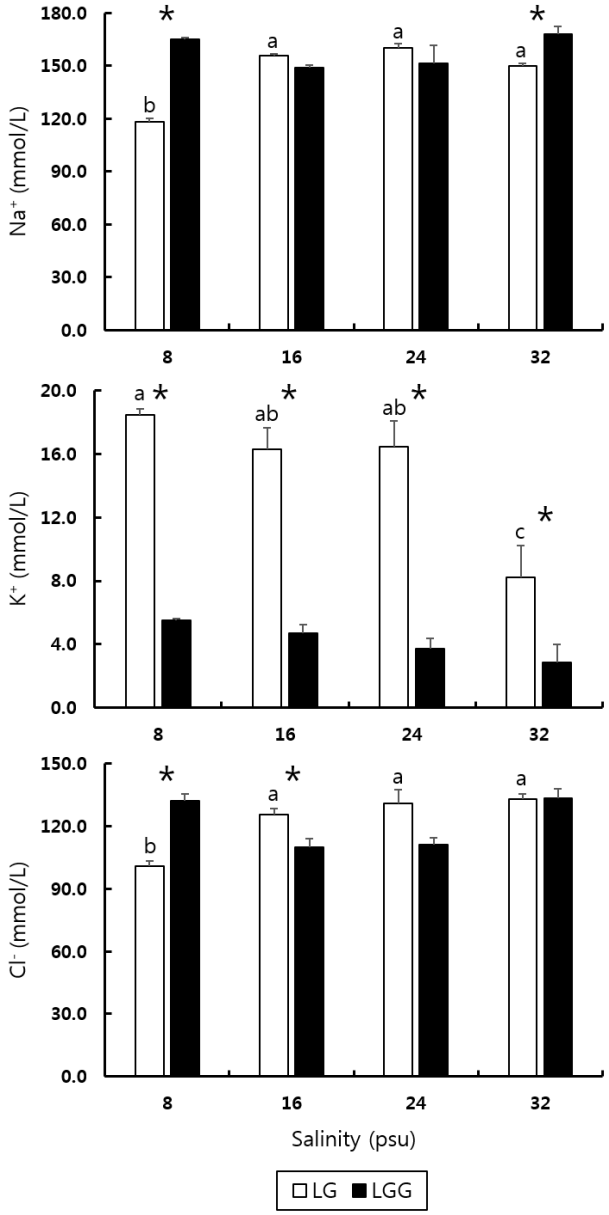


Fig. 3. Variations in plasma Na^+, K^+ and Cl^- concentrations in juvenile longtooth grouper (LG) and hybrid grouper (LGG) at different salinities. Different superscripts indicate significant differences at different salinities ($P < 0.05$). Asterisks show significant differences between juvenile longtooth grouper (LG) and hybrids (LGG) at each salinity ($P < 0.05$).

The plasma osmolality ranged from 360.3 ± 0.5 to 457.0 ± 0.1 mOsm/kg in the longtooth grouper and from 432.5 ± 25.5 to 441.8 ± 13.7 mOsm/kg in the hybrids, indicating no significant differences among the experimental groups (data not shown).

Discussion

Salinity variations are often observed in fish farms located in and around large estuaries during monsoons and flooding. Euryhaline fish can be farmed under a large range of salinities. This is advantageous not only to the farmed fish themselves, but also to the farm businesses. However, variable salinities may impose stress on farmed fish, causing slower growth, diseases, and even death in the long term. The tolerable and optimal range of salinities varies depending on fish species, and within a species, salinity tolerance may differ due to size, health conditions, and other environmental factors. Fish sometimes search for different salinity conditions at different life stages depending on their biological characteristics. Fish species like the Serranidae, which spend their early life in coastal or estuarine areas and then migrate offshore as they grow, are highly tolerant of salinity in their early stages but become gradually less tolerant as they mature (Lim et al., 2016).

The average initial size of the longtooth grouper and hybrid juveniles in this experiment was 10.7 ± 0.3 g and 20.3 ± 0.7 g, respectively, which increased to 18.2 ± 0.1 g and 58.9 ± 1.0 g, respectively, at the end of the experiment. Given the initial size difference between the two species, it cannot be said unequivocally that the hybrids exhibited faster growth than the longtooth grouper. However, considering that the two species hatched simultaneously, and the significant difference in weight gain between the two species during the 40-day rearing period (170% weight gain in longtooth grouper vs. 290% in the hybrids), we can conclude that the hybrids grew faster than the longtooth grouper. Consequently, it appears that juvenile longtooth grouper are better adapted to around 24 psu salinity than to natural seawater (32 psu) or salinities below 16 psu. This result is consistent with Boeuf and Payan (2001), who suggested that juvenile marine fish grow better at medium salinities. This could be because osmotic potential similar to that of the fish's body reduces energy consumption for osmotic regulation, thus providing more energy for growth. Our results are also supported by previous studies showing that serranid fish are better adapted to brackish water with lower salinities than to offshore waters (over 30 psu) during their early life. Meanwhile, the hybrid juveniles also showed the highest growth performance at 24 psu, but this result did not differ significantly from the fish in the other salinity groups. The hybrid juveniles did not show significant differences in weight gain among the experimental groups, apart from the 16 psu group. The results indicate that, as seen in the longtooth grouper, osmotic potential of culture water which is similar to that of the fish's body fluid supports growth, and also suggests that the hybrid has a larger range of salinity tolerance than the longtooth grouper. Longtooth grouper experienced mortality in the 8 psu tank, while the hybrids had no mortality in the lower salinity groups throughout the experiment, indicating that the hybrid is more tolerant of low salinity. Growth and survival data were very similar to results of other studies using hybrids of *E. lanceolatus* and either *E. akaara* or *E. coiodes* (Sutthinon et al., 2014; Lim et al., 2016).

Table 3. Optimal salinities for *Epinephelus* species.

Grouper Species	Fish Size	Optimal Salinity (psu)	Reference
Brown-spotted grouper (*E. tauvina*)	Late-stage larvae	25	Akatsu et al. (1983)
Malabar grouper (*E. malabaricus*)	Newly hatched larvae	8~24	Parado-Estepa (1991)
Orange-spotted grouper (*E. coioides*)	Newly hatched larvae	16~24	Toledo et al. (2002)
Dusky grouper (*E. marginatus*)	48.13 g	35	Lo´pez and Castello´-Orvay (2003)
Orange-spotted grouper (*E. coioides*)	56 g	12~18	Su-Jiu et al. (2011)
Giant grouper (*E. lanceolatus*)	1.56~1.69 g 39.18~67.66 g	20 10~30	Singhabun and Kummee (2013)
Hybrid grouper (*E. coioides*× *E. lanceolatus*)	23.49 g	10~30	Sutthinon et al.(2014)
Red spotted grouper (*E. akaara*)	8.3 g	16~24	Lim et al. (2016)
Hybrid grouper (*E. akaara* × *E. lanceolatus*)	10.0 g	16	
Longtooth grouper (*Epinesphelus bruneus*)	10.7 g	24	Present study
Hybrid grouper (*E. bruneus*× *E. lanceolatus*)	20.3 g	16~32	

As one of the known stress factors for reared fish, salinity variation increases plasma cortisol levels (Perry and Froese, 1993; Min et al., 2006; Lim et al., 2016). The increased cortisol levels work in fish blood and tissue to boost heart-beat, oxygen consumption, and energy mobilization and eventually disrupts the water and ion equilibrium (Eddy, 1981; Carmichael et al., 1984; McDonald and Milligan, 1997). This is why plasma cortisol and glucose are used as indicators of fish stress (Wedemeyer and Yasutake, 1977). Plasma levels of lactic acid, lipids, electrolytes, total protein, hemoglobin (Hb), hematocrit (Ht), and liver glycogen are also used as indicators of fish stress and fish health (Wedemeyer and Mcleay, 1981; Schreck, 1982). In our study, the blood cortisol level tended to increase at lower salinities in both longtooth grouper and hybrids. However, considering that the increased cortisol level rapidly stabilizes over time (Pickering and Pottinger, 1989; Tsui et al., 2012), we can't simply conclude that cortisol values measured for more than 30 days after directly represent the stress response in fish to changes in salinity. Therefore, it is thought that a higher plasma cortisol level at 8 psu represents a low chronic stress response that the fish uses to adapt to low salinities. In addition, given that normal fish have 30-40 ng/mL blood cortisol under stable, non-stressful conditions (Wedemeyer et al., 1990), the cortisol level at 8 psu in our study, which was higher than in the control group but still below 40 ng/mL, cannot be viewed as a severe stress condition caused by low salinity. It is assumed that with a longer rearing period, the cortisol level may recover to the level of the control group, as shown by our previous study in euryhaline starry flounder (Lim et al., 2013).

Fish exposure to stress activates both the brain-sympathetic-chromaffin cell axis and the hypothalamic-pituitary-adrenocortical axis, rapidly releasing catecholamine and cortisol into the blood (Schreck et al., 1989). This eventually leads to gluconeogenesis and glucose excretion into the blood. Thus, plasma glucose and cortisol levels are elevated simultaneously (Barton and Iwama, 1991; Nolan et al., 1999). In our study, plasma glucose increased together with plasma cortisol in the longtooth grouper, but in the hybrid, plasma glucose was higher under 16 and 24 psu conditions, regardless of cortisol levels. It is likely that more energy was needed for unknown reasons, such as homeostasis and growth, than for gluconeogenesis, accelerating glucose synthesis.

To identify stress-induced physiological changes, we also measured plasma osmolality and Na^+, K^+, and Cl^- concentrations, as well as Ht and Hb to determine the capacity for supplying oxygen throughout the body. During osmotic regulation, exchanges between water and ions take place. In our study, the concentrations of Na^+, K^+, and Cl^- were not significantly different among the hybrid groups under different salinity conditions. for the longtooth grouper, the concentrations of Na^+ and Cl^- were significantly lower in the 8 psu group than in the other groups, and, the concentration of K^+ was significantly lower at 32 psu that at the other salinity levels. It is assumed that the former result might be caused by hyper-osmoregulation, as we showed in a previous study (Lim et al. 2013). It is generally known that freshwater and marine bony fish have plasma osmolalities ranging from 250 to 350 mOsm/kg and from 350 to 500 mOsm/kg, respectively. In our study, both the longtooth grouper and the hybrids were in a normal range of osmolality, and the levels were not significantly different among the experimental groups. Min et al. (2006) recorded that euryhaline black porgy (13.4 ± 0.9 cm, 41.1 ± 13.5 g) exhibited increases and decreases in osmolality within a normal range during domestication to freshwater, suggesting that the constant plasma osmolality of both longtooth grouper and the hybrids in our study may be possible because the fish adapted to lower salinities through osmotic regulation.

Our study revealed that both longtooth grouper and hybrid juveniles are highly adaptable to salinity, and the hybrid has a stronger salinity tolerance than the longtooth grouper. We found that the optimal salinity was 24 psu for longtooth grouper, while it was 16-32 psu for the hybrids. As the fish in this study experienced gradual adaptations to lower salinities and were then reared for a long period, the stress response to salinity variation cannot be determined from this study. To obtain clearer information on the salinity tolerance of both species, further research is needed on the physiological changes that result from rapid changes in salinity.

Our study suggests the salinity change is important to control growth of grouper, which can contribute to the aquaculture production increase of grouper. In addition, it will provide basic knowledge on the effect of environmental changes on fish physiology.

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